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REVIEW - FOCUS ISSUE ON PLANT HEALTH SUSTAINING MEDITERRANEAN ECOSYSTEMS

Plant microbiota: from model plants to Mediterranean crops

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Summary. Plants live in ecosystems where they interact with complex microbial communities instigating a wide range of relationships. These communities constitute the 'microbiota', a term initially coined to describe host-symbiont systems that has been extended to cover non-symbiotic, but mostly beneficial interactions. Through the development of innovative '-omics' technologies such as high-throughput sequencing, study of plant microbiota has advanced rapidly, allowing scientists to increase understanding of plant primary functions and how these can be positively impacted by microbes. In addition, basic knowledge of plant-microbe interactions offers novel potential applications for sustainable agriculture. This review outlines new concepts of the 'plant metagenome', then summarizes major advances related to plant root-associated microbial communities, from model plants, such as *Arabidopsis thaliana,* to important Mediterranean crops. Arbuscular mycorrhizal fungi are crucial components of root microbiota: they are acknowledged as relevant tools for improving plant mineral nutrition in agricultural environments. Particular attention is given to their impacts on plant hosts, particularly tomato, which has been widely used as valuable model, both in plant biology and crop sciences, due to its importance in Mediterranean agriculture.

Key words: arbuscular mycorrhizal fungi, metagenomics, plant root, sustainability, tomato (*Solanum lycopersicum*).

Introduction

In recent times, a large body of experimental evidence has demonstrated that in natural ecosystems, organisms do not face their lives alone, but interact with each other, often in intimate cooperation. These interactions have deep impacts on reciprocal survival and fitness. Microbes (archaea, bacteria, fungi, protists and viruses), collectively the 'microbiota', thrive in association with host plants (Bordenstein and Theis, 2015). The term microbiota originated from study of host-symbiont systems, and was coined in 2001 by Lederberg and McCray (2001) referring to the complex microbial communities that inhabit the human body. First pioneer microbiota studies started in 2008, on the human-associated microbiota when the Human Microbiome Project was funded in the USA by National Institutes of Health.

In recent years many microbiome studies have been carried out in human biology and biotechnology (Garrett, 2017). These approaches have also been followed in other biological disciplines, including plant biology and entomology, also extending to non-symbiotic interactions and greatly extending biological knowledge. As in humans, many other complex multicellular eukaryotes host microbiota, including ancient eukaryotes such as fungi (Desirò *et al.*, 2014).

While microbiota research has mostly focused on host-symbiont interactions, the term microbiota is now widely used to also describe non-symbiotic associations. For example, in molecular ecology, this research encompasses complex microbial assemblages that live in particular environments or niches. Microbiota is commonly used in connection with the concept of 'metagenomes', which indicates the full set of genomes within the microbiota. This latter definition was used to describe the genetic content of soil microbiota (Handelsman, 2008), although first attempts were also made by Venter *et*

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al. (2004) with the genome shotgun sequencing of the Sargasso Sea.

When host-symbiotic models are considered, many authors now suggest that the 'holobiont' theory should be taken into account (Bordenstein and Theis, 2015; Vandenkoornhuyse *et al.*, 2015). According to this concept, these host-microbial systems, being complex assemblages of diverse organisms (Bordenstein and Theis, 2015; Theis *et al.*, 2016), constitute unique biological entities, and are defined as 'metaorganisms' or holobionts (Rosenberg *et al.*, 2007). These associations, which are often not randomly located, are crucial for reciprocal performance and survival, are probably driven by evolution, and constitute the 'hologenome', a newly accepted level on which selection pressures operate. The hologenome is defined as the host genome plus the microbiome (Rosenberg and Zilber-Rosenberg, 2016), i.e. the full set of microbial genomes associated with the host. The 'holobiont' theory was first postulated by Margulis (1991) to describe the interaction between a host and its endosymbiont. Later the term became generalized to other symbiotic associations including plants and animals, and 'hologenome' is now adopted to include the amounts of genetic information of a host and its associated microbiota.

In contrast, according to other authors, the hologenome concept as a level on which natural selection operates, has been misinterpreted. One of the main assumptions of hologenome evolution is partner fidelity, since each system needs to evolve as a unit (Moran and Sloan, 2015; Douglas and Werren, 2016). The major objection to this assumption is that in many systems, partner fidelity is weak since host-associated communities can strongly vary within and between host generations (Douglas and Werren, 2016). Additionally, it has been shown that the genotype has minor roles in shaping the microbiota (Vandenkoornhuyse *et al.*, 2015), and mutualistic interactions with hosts are possible even without having undergone natural selection (Moran and Sloan, 2015).

According to Theis *et al.* (2016), the term 'metagenome' and 'hologenome' should not be considered as synonyms. 'Metagenome' should refer, in environmental genomics, to the sum of genetic information from an environmental sample, including the full set of genomes from environmentally identified entities (Figure 1). 'Hologenome' should be exclusively applied to host-symbiont interactions (Bordenstein and Theis, 2015). However, due to the criticism raised by Douglas and Werren (2016), it is important to note this definition may not always consider the 'hologenome' as a level of selection.

In recent years, the concept of metagenome has dominated different areas of biological sciences, from the human-associated gut microbiota, to deep-ocean invertebrates, and to plant-microbe interactions. One of the most significant recent achievements is knowledge from the Earth Microbiome Project, which defined common standards in bacterial and archaeal microbiome sample collection and sequencing, developed a reference catalogue of microbes and microbiomes on Earth (Thompson *et al.*, 2017).

These studies expanded in conjunction with the emergence of new generation DNA sequencing technologies and high-throughput sequencing (HTS). These allowed issues linked to unculturable microbes to be overcome (Loman *et al.*, 2012). Notwithstanding uncertainty in the use of terms, recent discoveries have opened new research fields and the new (sometimes still confused) terminologies mirror the need to explore novel scenarios.

The aim of the present review is to present an update on the microbial communities associated with model plants as well as with some Mediterranean crops. Special attention is given to arbuscular mycorrhizal fungi (AMF), which are relevant components of plant root microbiota and positively act with many Mediterranean plants, which are also well-studied crop models, such as olive, grapevine, winter wheat and tomato. While some current information provides detailed responses to the question, "*who is there?*", understanding the impacts of beneficial microbes on the biology of many crop plants offers some cues to the other key question, "*what are plant-associated microbes doing*?"

The plant host: multiple niches for multiple microbial communities

Like animals, plants interact with many microbes, which are often defined as a plant's second genome (Berg *et al.*, 2014). Plants offer several micro-environments, which can be supportive of microbial life, providing favourable biotic and abiotic conditions. Plant ectosphere and endosphere can be distinguished (Berg *et al.*, 2014; Vandenkoornhuyse *et al.*, 2015): ectosphere includes plant outer surfaces while endosphere includes inner tissues. Considering plant anatomy, we can discriminate below-ground from above-

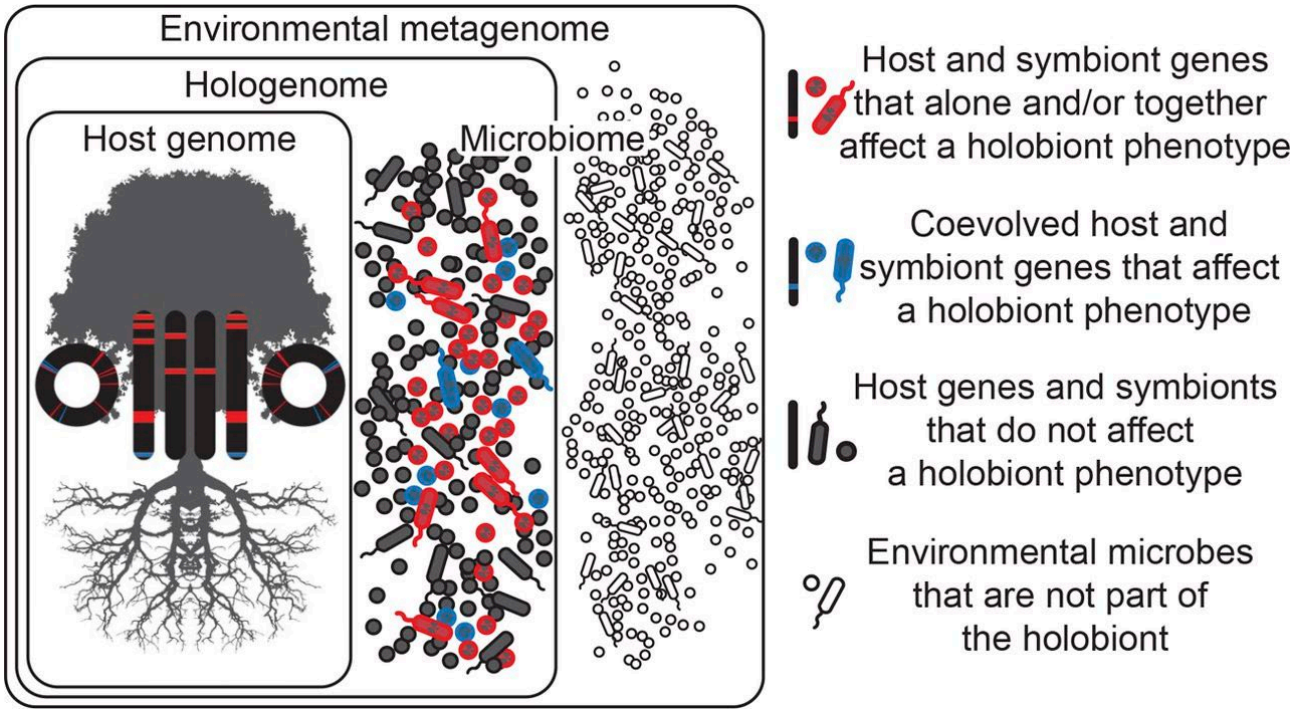

Figure 1. Level structure microbial communities from holobiont to environmental microbiota. Modified and here reproduced with the permission of the copyright holder from Theis *et al.* (2016).

ground micro-environments. For aerial plant parts, the phyllosphere (leaf), the anthosphere (flower), the carposphere (fruit) and the spermosphere (seed) can be distinguished (Berg *et al.*, 2014). Below ground level, at the interface between plant and soil, the root-microbiota complex occurs, which exists in several micro-habitats. Together with the phyllosphere, this complex is one of the most studied plant microbiota (Berg *et al.*, 2014). Being at the air-plant or soil-plant interfaces, both these microhabitats are exposed and influenced by air or soil microbiota, which includes phytopathogens (Weller *et al.*, 2002; Vorholt, 2012).

The plant root-associated microbiota: at the edge between plant and soil

At the root level, microbiota play crucial roles in modulating plant physiology and metabolism under different environmental conditions. These impacts may also influence plants at systemic levels, possibly shaping agronomically relevant traits. Understanding the mechanisms that plants modulate to control their microbiota, and functions activated by the microbiota, could be decisive for enhancing crop productivity (yield and quality) in sustainable agriculture (Berendsen *et al.*, 2012). For these reasons, microbial diversity associated with plant root compartments has been extensively studied since 2012, when the core root bacterial microbiota of *Arabidopsis thaliana* was defined (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012), in association with expansion of Next-Generation Sequencing (NGS) techniques (see Box).

Plant root microbiota are classified depending on the niches they occupy: the 'rhizosphere', located at the root periphery, includes the narrow soil layers surrounding the roots, the 'rhizoplane' is located at the root surface, and the 'endorhiza' (Berg *et al.*, 2014) is a compartment hosted within plant tissues (Heijden and Schlaeppi, 2015). Recent studies have demonstrated that the selection of plant root bacteria occurs at these three locations, originating a model that includes a host-driven selection of associated microbes from rhizospheres to endorhiza. This model was defined using plant models, including rice (Edwards *et al.*, 2015), maize (Peiffer *et al.*, 2013) and *Arabidopsis* (Schlaeppi *et al.*, 2014). According to this

BOX – Meta-omics to study plant microbiota

Recent next-generation sequencing (NGS) technologies, such as high-throughput sequencing (HTS), have shown that only 5% of whole plant-associated microbial diversity is culturable (Mendes *et al.*, 2013). Study of plant-associated biota using NGS technologies has become standard (Schlaeppi and Bulgarelli, 2015), due to cost-effectiveness, revolutionizing understanding of the ecology of specific microbial niches (rhizosphere *versus* bulk soil), and their functioning. Improvements from sequencing-by-synthesis, taken up by the Illumina company, have given increased throughput and greater sequencing depth at reduced cost. Third-generation single-molecule sequencing has recently become possible. This avoids PCR-based amplification (with its intrinsic biases), producing long reads but with less sequencing depth. Good examples are Single Molecule Real-Time (SMRT) Sequencing by Pacific Biosystems (PacBio sequencing), and Nanopore sequencing by Oxford Nanopore Technologies (Creer *et al.*, 2016). However, even if these techniques are appealing and promising for new applications in genomes assembly, they are still in their infancy, and major efforts are needed to reduce sequencing errors and cost, and increase the throughput. NGS sequencing based on Illumina technology is currently the state-of-art methodology (metagenomics, metabarcoding, transcriptomics and meta-transcriptomics), for studying microbiota diversity and understanding host impacts from associated microbes (Creer *et al.*, 2016). Metabarcoding has become a standard to characterize microbial community assemblages. This technique is based on targeted amplicon sequencing of selected, phylogenetically informative, marker genes (Schlaeppi and Bulgarelli, 2015; Creer *et al.*, 2016).

For Prokaryotes, the 16S ribosomal RNA gene was widely-used, since it contains slowly evolving sequence regions and many sequences are available in public databases. For Eukaryotes, the 18S rRNA gene is used with some exceptions. For example, internal transcribed spacer (ITS) profiling is used for fungal communities.

Together with the increase in microbial genome availability and advanced bioinformatics analyses, metabarcoding also allows definition of functions associated with identified organisms. This is performed by 'predictive metagenomics', which allows linking of phylogenetic marker information with reference annotated genomes, predicting functions that could be activated in particular systems (Langille *et al.*, 2013; Bulgarelli *et al.*, 2015). These approaches are currently only available for Prokaryotes, but will probably become available for Eukaryotes, such as fungi, due to recent increases in sequenced genomes (see the 1,000 fungal genome project at U.S. Department of Energy Joint Genome Institute (DOE JGI), http://1000.fungalgenomes.org).

For microbiota functioning, NGS approaches have increased knowledge of ecosystems, mostly using metagenomics in combination with other meta-omics tools. "Shotgun" metagenomics allows reconstruction of complete DNA information within environmental matrices or tissue samples. This provides knowledge of putative functions that can be activated. However, due to high cost and extensive computational requirements, this technique is often superseded by other approaches such as meta-transcriptomics (Bulgarelli *et al.*, 2013).

Illumina sequencing has always been optimized for genome-targeted transcriptomics, for human and plant systems (Conesa *et al.*, 2016). Large scale genome-wide analyses such as mRNA-seq can reveal the complete transcriptomic profiles of organisms living under specific treatments or environmental conditions. This provides understanding of the molecular basis of development or stress resistance. As for plant-microbiota systems, however, distinguishing microbiota from host functions remains difficult. Meta-transcriptomics is becoming increasingly productive, allowing understanding of functions activated by free-living and intimate host-associated microbial communities in different systems (Kopylova *et al.*, 2015).

Both approaches (genome-targeted transcriptomics and meta-transcriptomics) need to be better integrated. Within endosphere (e.g. endorhiza), due to the intimate spatial connections between hosts and microbes (as for human gut microbiota), host RNAs abundance, characterizing microbial functions is cost-expensive since it requires deep-sequencing approaches. In other cases, such as for the rhizosphere compartment, separate sequencing experiments are required to account for spatial separation of hosts from associated microbial communities, again requiring large financial inputs and time efforts.

scheme, enrichment steps occur both in rhizosphere and rhizoplane, and are controlled by root exudates (Chaparro *et al.*, 2014). Conversely, depletion/exclusion processes play roles only latterly in the rhizoplane, and markedly in the root endosphere where plant immune systems are likely to have crucial roles (Heijden and Schlaeppi, 2015). Lebeis *et al.* (2015) showed that *Arabidopsis* phytohormone mutants affected in salicylic acid synthesis and signalling, differently shaped their microbiota in comparison with wild-type plants.

Plant microbiota studies have demonstrated that the microbial diversity and physiochemical-edaphic traits in soils are the main drivers of below-ground plant microbiota assembly and function where the soil is probably more important than plant genotype (Bonito *et al.*, 2014; Vandenkoornhuyse *et al.*, 2015). This is defined as the "soil effect" (Alegria Terrazas

et al., 2016). Unlike the human gut microbiota, which is inherited vertically from mothers to their children, the root microbiota is re-established for each individual, since a seed germinates within the soil microbial bank available at the time of re-generation (Heijden and Schlaeppi, 2015; Bai *et al.*, 2015). However, recent works demonstrated the existence of a seed microbiota, which is likely inherited vertically across successive plant generations, but our knowledge on this topic remains limited (Shade *et al.*, 2017). On the other hand, the plant host genotype is the second determinant of root microbiota assemblages (Bouffaud *et al.*, 2014). Zgadzaj *et al.* (2016) showed that genetic plant determinants also affect root nodule host-microbe symbioses. They demonstrated that mutations in *nfr5*, *nin* and *lhk1* genes, which are involved in nodulation in *Lotus japonicus*, lead to order-level alterations in bacterial communities when compared to wild-type plants. The relevance of host genotype was also clearly shown when the bacterial root microbiota was investigated by comparing wild and domesticated barley (Bulgarelli *et al.*, 2015). Some bacterial families (Rhizobiaceae, Flavobacteriaceae, and Comamonadaceae) were dominant in the root-associated microbiota of *Hordeum vulgare,* and significant effects on bacterial community diversity was detected among the cultivars, representing taxa associated with barley domestication. To better understand the role of host phylogeny on plant microbiota, Yeoh *et al.* (2017) investigated the root microbiota of many plant phyla, including lycopods, ferns, gymnosperms, and angiosperms, across a tropical soil chronosequence using 16S rRNA gene amplicon profiling. They confirmed the role of soil type as the first driver of microbial diversity, but also detected a correlation with plant phylogeny. They identified 47 common bacterial genera, including *Bradyrhizobium*, *Rhizobium*, and *Burkholderia*, as well as some uncharacterised lineages. These taxa could constitute an evolutionarily conserved core root microbiome at the specific tropical site. The working hypothesis is that a core root microbiome has evolved with terrestrial plants over their 400-million-year history (Yeoh *et al.*, 2017).

These investigations have allowed the identification of the main bacterial taxa that live in association with model and crop plants, and with basal plant clades, offering novel evolutionary perspectives. Table 1 outlines a summary of the current knowledge. This information mostly considers plant-associated prokaryotes, while other relevant taxonomic groups have been neglected. Fungi are one of these (Guttman *et al.*, 2014), exerting crucial functions and being one of the more abundant plant-associated microbiota. Studies focused on the taxonomic profiles of selected fungal taxa such as mycorrhizal fungi (Davison *et al.*, 2015), as well as on whole communities, have been recently outlined (Porras-Alfaro and Bayman, 2011; Toju *et al.*, 2013). These studies highlighted that root compartments are dominated by Ascomycota and Basidiomycota (Hacquard, 2016). Among them, Pleosporales, Agaricales, Sordariales, Hypocreales and Xylariales are the most represented fungal orders (Porras-Alfaro and Bayman, 2011). However, deep knowledge of complete root-associated fungal communities in model and crop plants is still lacking, and only a few attempts have been made using high-throughput sequencing on model plants, such as with sugarcane (Souza *et al.*, 2016), rice (Wang *et al.*, 2016), wheat (Rascovan *et al.*, 2016) and *Arabis alpina* (Almario *et al.*, 2017) (see Table 1).

Thanks to the success of NGS and the selection of suitable marker regions and primers (Lindahl *et al.*, 2013), recent studies have provided more detailed contributions on bacterial and fungal communities and their interactions. As an example, Sun *et al.* (2017) investigated the intra-annual variability and decadal scale recovery of bacterial and fungal communities in a chronosequence of reclaimed mined soils to quantify the microbial abundance, richness, β-diversity, and taxonomic composition. They detected contrasting dynamics of bacteria *versus* fungi in the chronosequence, leading to the hypotheses that: 1) the faster growth rates for bacteria lead to increased intra-annual variability; 2) fungi show a greater tolerance to environmental changes than bacteria; and 3) plant species assemblage has a stronger influence on fungal than bacterial communities. Overall, experimental evidence indicates that fungal communities are more subjected to variations linked to stochastic factors and biogeography than bacterial communities, and the assemblage is likely to be driven mainly by the plant compartment (Hacquard, 2016).

These new data indicate that existing information on plant microbiota must be complemented by considering the diverse microbial groups: in this context co-occurrence network analysis provides novel tools to generate testable hypotheses about microbe interactions, and also indicates candidate microbes that may affect plant health. As demonstrated for wheat roots (Poudel *et al.*, 2016), microbiome network stud-

Table 1. Taxonomy of some available root-associated microbiota to model and crop plants by compartment (rhizosphere, root-endosphere) investigated by NGS studies. Over-represented phyla and most abundant taxa are listed (*class* to *genus* as available in each reference). Where taxonomic profiles were overlapping the composition was listed once. In same cases the analysed root compartments were not specified and was defined generically as 'root-associated'.

Kingdom	Plant host	Compartment	Represented group	Most abundant taxa	References
Bacteria					
	Arabidopsis thaliana (L.) Heynh. (thale cress)	Rhizosphere	Acidobacteria, Proteobacteria, Planctomycetes, Actinobacteria	Streptomycetaceae, Comamonadaceae, Pseudomonadaceae, Bradyrhizobiaceae, Flavobacteriaceae, Sphingomonadaceae, Phyllobacteriaceae, Xanthomonadaceae	(Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012)
		Root endosphere	Proteobacteria, Actinobacteria, Chloroflexi, Bacteroidetes, Firmicutes	Flavobacteriaceae, Streptomycetaceae, Comamonadaceae, Oxalobacteraceae, Rhizobiaceae, Methylobacteriaceae, Pseudomonadaceae, Moraxellaceae, Sphingomonadaceae, Burkholderiaceae	
	Solanum lycopersicum L. (tomato)	Rhizosphere	Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, Acidobacteria, Planctomycetes, Verrucomicrobia	Sphingomonadaceae Chitinophagaceae, Microbacteriaceae, Bacillaceae, Strepromycetaceae, Flavobacteraceae, Xanthomonadaceae, Mycobacteriaceae, Rhizobiaceae, Psudomonadaceae, Burkholderiales,	(Ofek *et al.*, 2014; Lee *et al.*, 2016; Larousse *et al.*, 2017; Tian *et al.*, 2017)
	Vitis vinifera L. (grapevine)	Root-associated (unspecified)	Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria	Xanthomonadales, Rhizobiales, Saprospirales, Cytophagales, Actinomycetales	(Zarraonaindia *et al.*, 2015)
	Oryza sativa L. (rice)	Rhizosphere	Bacteroidetes, Firmicutes, and Proteobacteria, Acidobacteria, Planctomycetes, Chloroflexi, Verruc omicrobia,Gemmatimonadetes	Rhodocyclaceae, Comamonadaceae, Pleomorphomonas	(Edwards *et al.*, 2015)
		Root endosphere	Proteobacteria, Chloroflexi, Bacteroidetes, Fibrobacteres, Spirochaetes	Rhodocyclaceae, Comamonadaceae, Pleomorphomonas, Methylocystaceae, Kineosporiaceae, Myxococcaceae, Methanobacteriaceae	
	Hordeum vulgare L. (barley)	Rhizosphere	Actinobacteria, Bacteroidetes, Proteobacteria	Rhizobiaceae, Comamonadaceae, Oxalobacteraceae, Flavobacteriaceae	(Bulgarelli *et al.*, 2015)
		Root endosphere	Actinobacteria, Bacteroidetes, Proteobacteria	Streptomycetaceae, Rhizobiaceae, Comamonadaceae, Oxalobacteraceae, Flavobacteriaceae	
	Zea mays L. (maize)	Rhizosphere	Actinobacteria, Proteobacteria	Burkholderiales, Oceanospirillales, Sphingobacteriales, Pseudomonadaceae	(Peiffer *et al.*, 2013; Ofek *et al.*, 2014)

(Continued)

Table 1. (Continued).

Kingdom	Plant host	Compartment	Represented group	Most abundant taxa	References
Fungi					
	Arabis alpina L. (alpine rock-cress)	Rhizosphere	Zygomycota Ascomycota	Mortierellales (*Mortierella* sp.), Pleosporales (*Leptosphaeria* sp.), Hypocreales (*Blonectria* sp.), Sordariales (*Chaetomium* sp.)	(Almario *et al.*, 2017)
		Root endosphere	Basidiomycota Ascomycota	Ceratobasidiaceae, Helotiales (*Tetracladium* sp., *Cadophora* sp.), Cantharellales, Pleosporales, (*Alternaria* sp.), Hypocreales, (*Dactylonectria* sp.)	
	Oryza sativa L. (Rice)	Root endosphere	Basidiomycota Ascomycota	*Hypocreales, Eurotiales, (Penicillium sp., Aspergillus sp.), Pleosporales, Trichosporon* sp., *Pestalotiopsis* sp., *Fusarium* sp., *Verticillium* sp., *Cryptococcus* sp.,	(Wang *et al.*, 2016)
	Saccharum officinarum L. (Sugarcane)	Root endosphere and rhizosphere	Glomeromycotina, Basidiomycota, Ascomycota	Glomerales, (Glomeraceae), Polyporales, (Meruliaceae), Cantharellales, (Sistotremataceae), Microascales, (Ceratocystidaceae, Chaetosphaeriaceae)	(Souza *et al.*, 2016)
	Triticum aestivum L. (Winter Wheat)	Root endosphere and rhizosphere	Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota, Glomeromycotina	*Candida* sp., *Trichosporon* sp., *Dipodascus* sp., *Blastobotrys* sp., *Bensingtonia* sp., *Blumeria* sp.	(Granzow *et al.*, 2017)

ies may provide guidance for selecting microbial taxa to be used for biological control, biofertilization, and microbe-associated crop breeding.

Rhizosphere, rhizoplane, endorhiza: different niches host different microbial communities

The rhizosphere is one of the most intricate ecosystems (Raaijmakers and Mazzola, 2016): it plays pivotal functions in nutrient solubilization and uptake by plants, as well as in protection against soil-borne pathogens (Berendsen *et al.*, 2012). This compartment hosts one of the most biodiverse microbiota known (Rosenberg and Zilber-Rosenberg, 2016), with more than 30,000 prokaryotic species and a mean density of 10^{11} cells per gram, together constituting a genome larger than that of the plant hosts (Berendsen *et al.*, 2012).

Soil surrounding the roots is largely influenced by plant exudates. This phenomenon, acknowledged by many authors, was defined as the 'rhizosphere effect' and strictly depends on root exudates. Plants invest up to 40% of photosynthetic products to produce active phytochemicals (Badri *et al.*, 2009) such as amino acids, phenolics, sugars or sugar alcohols, that are secreted by roots. These rhizodeposits greatly influence microbial communities in root-surrounding soil since their amounts are correlated with microbial abundance (Chaparro *et al.*, 2014). These molecules allow plant to select and shape their root-associated microbiota, stimulating or repressing members of the microbial communities (Doornbos *et al.*, 2012). As an example, some chemotactic bacteria can move toward a gradient of organic substrates such as root exudates (Miller *et al.*, 2009). As a result, the microbial density in rhizosphere compartments is known to be much greater than in bulk soil, even if the recruited communities are less diverse (Bulgarelli *et al.*, 2012).

Other evidence suggests that the 'rhizosphere effect' is weak since taxonomical diversity in this compartment is often not distinguishable from that of bulk soil and weakly associated to host determinants (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012; Schlaeppi *et al.*, 2014). Moreover, an increasing body of evidence is showing that host-microbe interactions and microbe-microbe interactions can both actively shape rhizosphere microbiota (Cardinale *et al.*, 2015; Hacquard *et al.*, 2015). This results from several mechanisms, including mutualism, commensalism, competition, parasitism or amensalism (Berendsen *et al.*, 2012). Furthermore, Rosenberg and collaborators (2009) found that predation of bacteria by protozoa can modify overall rhizosphere community assemblages.

Taxonomic assembly of rhizosphere microbiota has been closely studied, and many studies showed that fungi, bacteria, archaea, algae, viruses, protozoa, nematodes, oomycetes and arthropods are present (Mendes *et al.*, 2013) in rhizosphere. This biodiversity confirms that the rhizosphere is the crossing points of complex food webs, which are based on nutrients released by plant roots (Buée *et al.*, 2009; Raaijmakers and Mazzola, 2016). As already commented, only bacterial diversity has been well-characterized while other groups such as fungi, algae or viruses have been more rarely considered. These other components are also predicted to have considerable relevance for plant fitness. For example, fungi represent a large component of rhizosphere biomass (Hannula *et al.*, 2010), and many studies have demonstrated that, beside bacteria, fungi also metabolize significant amounts of rhizodeposits (Broeckling *et al.*, 2008; Buée *et al.*, 2009; de Graaff *et al.*, 2010). Rhizosphere bacterial communities were found to be dominated by Proteobacteria, Acidobacteria, Bacteroidetes and Planctomycetes, both in herbaceous (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012) and woody plant species (Uroz *et al.*, 2010). A comprehensive study on 35 different taxonomic orders of dicots and monocots revealed that among these microbes, Proteobacteria are the most abundant (Hawkes *et al.*, 2007; Hacquard *et al.*, 2015).

Rhizoplane is a separate compartment from rhizosphere and this includes all root surfaces where microrganisms live attached (Vandenkoornhuyse *et al.*, 2015). Fluorescence *in situ* hybridization (FISH) has revealed that the rhizoplane spatial distribution is not homogeneous, but colonization preferentially occurs at specific points where the access to individual roots is easier, such as root tips and lateral root cracks (Hardoim *et al.*, 2008). Rhizobia are a good example of preferential colonization: they firstly colonize host rhizoplanes concentrating on the root hairs where they then find access to root tissues (Oldroyd, 2013).

The rhizoplane is often defined as the key point for root tissue colonization. Microscopy and sequencing techniques have revealed that phyla abundant in rhizoplane are also dominant in endorhiza compartments (Bulgarelli *et al.*, 2012). The root surface epiphytic microbiota must be considered as a tran-

sitional state that preludes endosphere colonization. For these reasons, study of the rhizoplane in plant microbiota research has recently been highlighted.

Beside bacteria, mycorrhizal fungi are well-recognized components of rhizoplane compartments (see Part 2). Irrespective of their colonization strategies, which can be inter- or intra-cellular (Bonfante and Genre, 2010), these fungi commence symbiotic phases with specialized contact with the root epidermal cells. Ectomycorrhizal fungi (EMF), which mostly interact symbiotically with tree roots (Cairney, 2011), are particularly abundant, since they develop complex structures ("mantle") starting from host rhizoplane. However, rhizoplane microbiota can also hold important functions for plant health not necessarily linked to symbiotic interactions. Among rice rhizoplane colonizing bacteria, for example, several isolates were found to be efficient in phosphate solubilization or auxin production, leading to plant growth stimulation (Mwajita *et al*., 2013).

Most land plants host extensive colonization of root internal tissues, where they specifically select the hosted communities (Vandenkoornhuyse *et al*., 2015). Community assemblages are very different from those of the rhizosphere or surrounding bulk soil, and diversity is less (Bulgarelli *et al*., 2012; Schlaeppi *et al*., 2014). Root endosphere is colonized by symbionts such as ECM fungi, obligate symbionts, such as arbuscular mycorrhizal fungi (AMF), or facultative transient endophytes, like *Trichoderma* or *Piriformospora indica* (Andrade-Linares and Franken, 2013). One of the most represented components are AMF (see part 2) together with other fungal taxa, bacteria and archaea (Vandenkoornhuyse *et al*., 2015). Different from AMF (Figure 2), all the endorhizal microbes are hardly distinguishable at morphological levels, and are identifiable only using the recent metabarcoding and metagenomic technologies. Among bacteria, Actinobacteria were found to be most abundant, and this group includes several producers of antimicrobial compounds, such as the Streptomycetaceae (Mendes *et al*., 2011).

The scenario emerging from all these studies reveals progressive impacts of plant genotype on the microbial communities. In the rhizosphere, the soil is probably the main driver regulating microbial diversity, but first physical contact between plant cells and microbes occurs in the rhizoplane. Recognition events and defence mechanisms are triggered at this moment and at this location, strictly regulating the further colonization events of root endosphere. However, knowledge of the shifts in prokaryotic and eukaryotic microbial communities from the rhizosphere to the endosphere remains to be fully determined.

Functioning and host impacts of plant root-associated microbiota

In addition to description of microbial diversity, the advancing of metagenomic and metatranscriptomic approaches has allowed the functions held or activated by microbiota to be determined. This provides knowledge of links between microbial diversity and functions, by comparing observations with theories recently postulated (Vandenkoornhuyse *et al*., 2015): the **key species hypothesis** (Paine, 1969), by which functions are linked to single components of the microbiota, and the **functional redundancy hypothesis** (Walker, 1992), which assumes that organism diversity contributes to a given function.

Because plants have sessile lifestyles, they can shape their associated microbiota, and recruit protective microbes when attacked by pathogens or insects, or adapt to environmental stresses (Berendsen *et al*., 2012). These changes may also mirror domestication processes. Using metagenomic approaches, Bulgarelli *et al.* (2015) demonstrated that traits related to nutrient mobilization, phage interactions, pathogenesis and secretion were enriched in the taxa associated with barley roots. Protein families related to these traits revealed evidence of positive selection among the barley lines. These results indicate that microbe-microbe interactions also shape the microbiota assemblages in plant root compartments during domestication events. On the other hand, microbes increase the defensive capacity of plants against pathogens by modulating host immunity, inducing systemic plant resistance or improving plant nutrition and growth (Müller *et al*., 2016). These microbiota-extended plant traits are mainly linked to rhizosphere inhabitants, but also some endosphere organisms may have important roles, for example AMF.

As largely acknowledged, microbes associated to the root compartments can be functionally divided into three main types: plant pathogenic, beneficial, and human pathogenic (Raaijmakers and Mazzola, 2016). Here, only the beneficial soil microbes will be considered. Plant pathogens are present among the plant-associated microbes, but they represent the specific focus of a several recent reviews (Brader *et al*.,

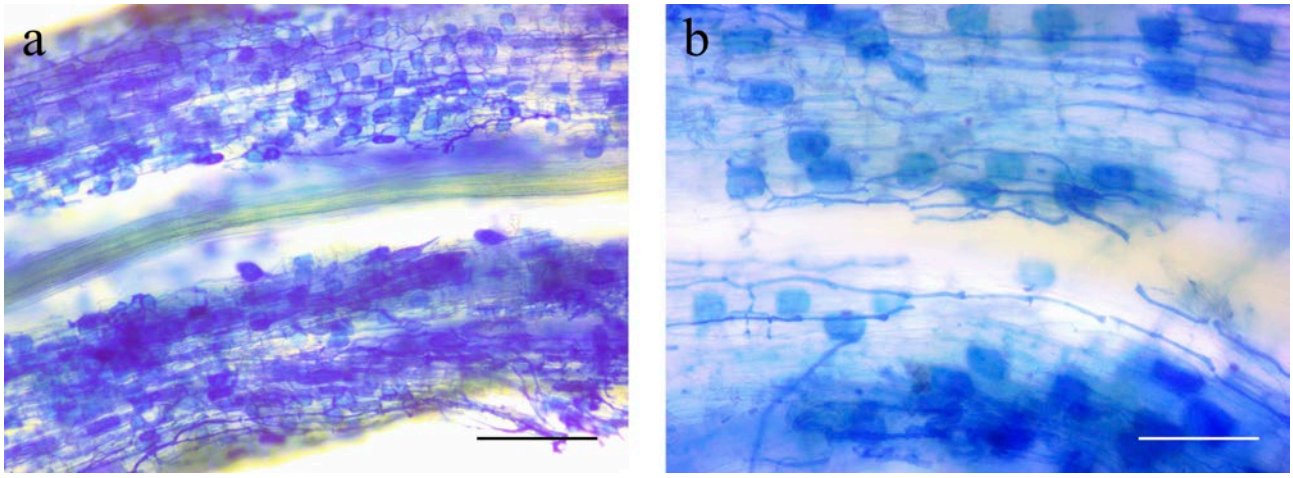

Figure 2. Micrograph showing AMF colonizing *Olea europaea* cv. Frantoio (olive) roots collected in the field and stained using methyl-blue (a-b). Scale bars: a = 500 μm; b = 200 μm.

2017; Möller and Stukenbrock, 2017; Naseem *et al.*, 2017; Peyraud *et al.*, 2017).

In the view of the holobiont theory, facilitation (positive beneficial interaction) is considered the main driver of plant-associated microbial diversity. Microbiota genes can be considered as extensions of plant genomes, providing local adaptations to environmental conditions (Vandenkoornhuyse *et al.*, 2015) (see Figure 1). This is further supported by the fact that the main driver of root microbiota assemblages is the soil, rather than plant genotype (Bulgarelli *et al.*, 2012).

Classical studies using one-to-one interaction experiments under laboratory conditions demonstrated that several root-associated microbial taxa (both at endosphere and rhizosphere level) exert beneficial effects on their plant hosts. Among these, the plant growth-promoting rhizobacteria (PGPRs) and AMF are well-known components. Both are known to provide their hosts indirect pathogen protection and enhanced nutrient acquisition (Bonfante and Genre, 2010; Thomashow and Bakker, 2015). Early evidence demonstrated that variation in rhizosphere assemblages occurs upon pathogen infection because of induced root secretion of antimicrobial compounds. These changes in root exudation have been demonstrated to be linked with phytohormones such as jasmonic acid and salicylic acid (Doornbos *et al.*, 2012; Lebeis *et al.*, 2015). Several studies have shown that plant immune systems have key roles in mediating interactions with soil microbes, including beneficial interactions. Plant immunity, which mainly regulates plant-pathogen interactions, is at the base of this system. Many soil microbes can boost plant defence-related traits at systemic levels, with induced systemic resistance (ISR). Non-pathogenic rhizobacteria activate ISR through signalling pathways that overlap with pathways activated by pathogen or herbivore attack such as the Plant-triggered immunity (PTI) (Conrath, 2006; Pieterse *et al.*, 2014). These responses are probably not associated with direct defence activation, but with a priming that speeds and strengthens the activity of defence-related genes (Van Wees *et al.*, 2008; Pieterse *et al.*, 2014). ISR responses elicited by soil microbes may include production of salicylic acid (SA), which is independent of and mainly driven by other hormones such as jasmonate and ethylene (Pieterse *et al.*, 2014). SA-dependent plant responses to microbes have been detected when associated with SA-producing organisms, such as *Trichoderma*, some *Pseudomonas* strains and other bacteria. However, in these cases the triggered plant responses probably follow the systemic acquired resistance (SAR) signalling pathway, which involves the activation of PTI and effector-triggered immunity (ETI) mechanisms (Pieterse *et al.*, 2014) (Figure 3).

The involvement of plant immune systems is probably even greater for microbial establishment in the plant endosphere. In this case, it is likely that microbe associated molecular patterns (MAMPs) and damage

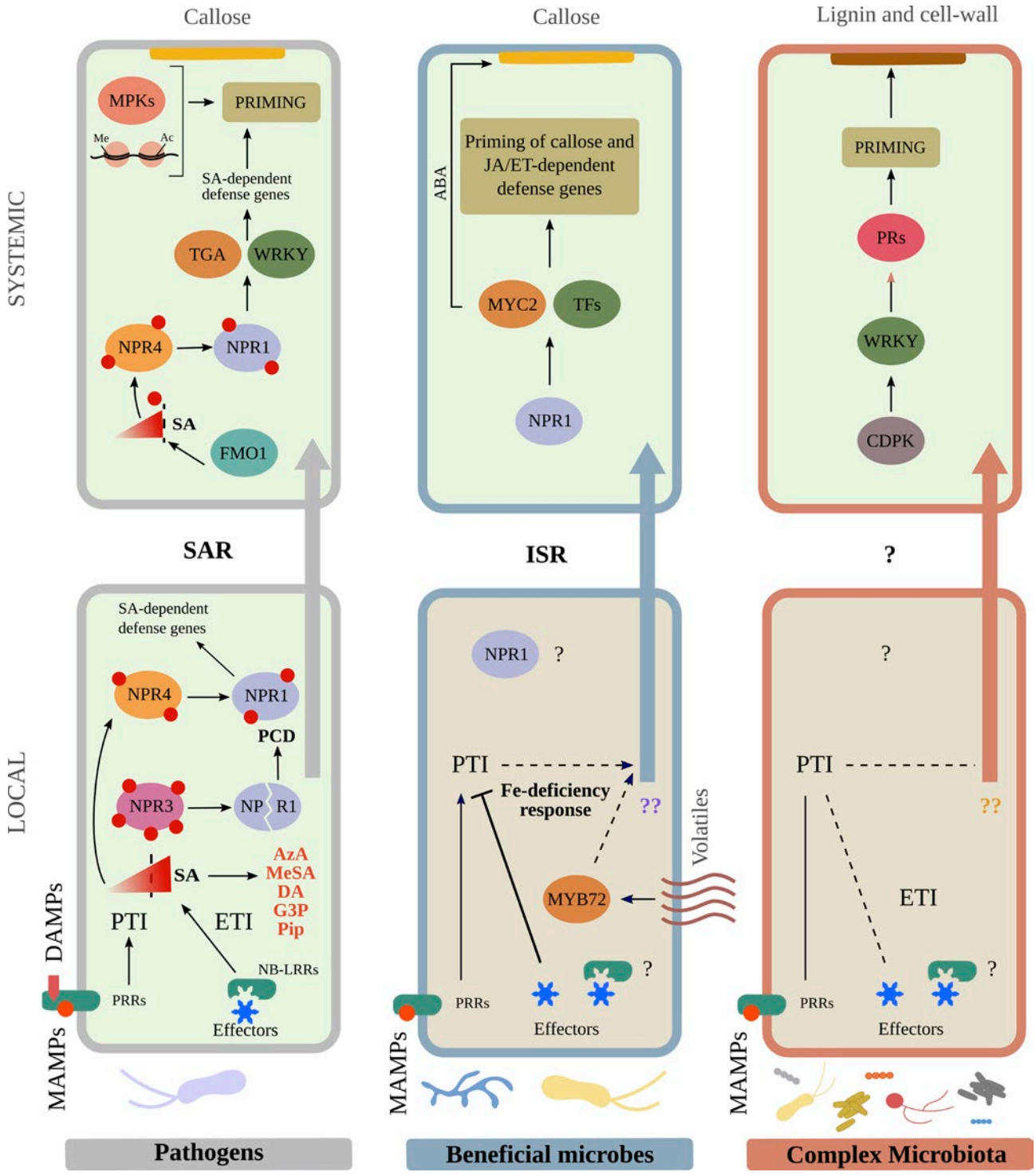

Figure 3. Plant immunity interaction mechanisms involved in plant-pathogen (left), plant-beneficial microbe (middle) and complex soil microbiota (right) interactions, according to the models proposed by Pieterse et al. (2014) and Chialva et al. (2018).

associated molecular patterns (DAMPs) are bypassed to allow microbial accommodation. Furthermore, mechanisms of association, attraction and recognition should be universal across plant and microorganism species (Vandenkoornhuyse *et al.*, 2015). For these reasons, it was proposed that the common symbiosis pathway (CSP) could have a role (Venkateshwaran *et al.*, 2013). Supporting this suggestion, it was shown that lipochitooligosaccharides (LCOs), a class of signal molecules, which are involved in plant-rhizobial and plant-mycorrhizal fungus interactions (Maillet *et al.*, 2011), can also stimulate plant growth and seed germination (Wang *et al.*, 2012).

Neutral commensal microbes have also been shown to elicit plant immunity responses. A recent study by Vogel *et al.* (2016) demonstrated that *Arabidopsis* plants inoculated with *Methylobacterium extorquens*, a leaf commensal, reprogrammed their transcriptional activity triggering defense-related genes overlapping with those elicited by the pathogen *Pseudomonas syringae* DC3000. However, in many other cases, inoculation of other commensal bacterial such as *Pseudomonas syringae* isolates on *Arabidopsis*, did not cause any relevant changes at the transcriptomic level (Vogel *et al.*, 2016). Full comprehension of the roles of root and leaf commensals has yet to be achieved, and further research is required, probably considering other model plants.

It is well known that root-associated bacteria also help plants to tolerate some abiotic stresses, especially under extreme environmental conditions (Mendes *et al.*, 2013; Soussi *et al.*, 2016). Particularly in the rhizosphere, many bacterial isolates were found to enhance osmotic stress tolerance under salt stress (Upadhyay *et al.*, 2009), transient drought stress (Mayak *et al.*, 2004) and flooding conditions (Ravanbakhsh *et al.*, 2017). Other bacterial isolates were found to provide tolerance at low and freezing temperatures (Mishra *et al.*, 2012), under low pH conditions or in the presence of soil pollutants (Mendes *et al.*, 2013).

To date, very few attempts have been made to understand the effects of complex and interacting microbiota on plant health using multidisciplinary and multi-omics approaches (Chialva *et al.*, 2018).

Disease suppressive soils support redundancy theory

New knowledge is supporting the redundancy theory focusing on beneficial microbe consortia. Some experiments show that, at least for PGPRs, host fitness is increased when these microbes are applied in consortium (Hays *et al.*, 2015). These data support the idea that microbiota components accomplish network modes of activity (Gopal and Gupta, 2016).

Disease-suppressive soils are good examples of these phenomena. These soils can reduce pathogenic attacks of plants as a result of changes in soil microbial composition (Mendes *et al.*, 2011). Disease suppression in soils is explained by two potential mechanisms: 'general' or 'specific' suppression (Berendsen *et al.*, 2012; Raaijmakers and Mazzola, 2016). 'General suppression' is a universal feature that acts in all soils. Most soil-borne plant pathogens can grow saprotrophically in the soil before they attack living plants to cause disease outbreaks, and in soil they are subjected to competition for resources (mostly plant derived nutrients). This mechanism of suppression is linked to total microbial activity, since under these situations microbe to microbe competition takes place, and some pathogens may be less efficient than others for resource uptake.

The mechanism of 'specific suppression' is attributable to specific soil enriched microbial communities. This effect is stronger and more effective than general suppression. A good example is *Fusarium* wilt suppressive soils in which the pathogen is specifically suppressed by antagonistic *Fusarium* strains that compete for carbon, and by *Pseudomonas* spp. that produce the antimicrobial compound phenazine (Mazurier *et al.*, 2009; Pieterse *et al.*, 2014). Disease-suppressive soils are found worldwide and are examples of co-evolution between plants and beneficial microbes. They often originate after prolonged periods of monocrop culture (Pieterse *et al.*, 2014), allowing a particular plant species to recruit and enrich specific beneficial components with biocontrol activity over time (Berendsen *et al.*, 2012). On the other hand, since soils with specific suppressiveness are widespread, similar biocontrol strains are globally present in different soils, and may be recruitable by different plant species.

Several microbial taxa have been found to be linked with disease suppression, such as *Trichoderma*, *Fusarium*, *Xylaria*, *Streptomyces*, *Bacillus*, and *Actinomyces* spp. (Mendes *et al.*, 2011; Berendsen *et al.*, 2012; Penton *et al.*, 2014). These microbes compete for space and nutrients, and also display features such as hyperparasitism and production of secondary metabolites, and may also elicit ISR in their hosts (Pieterse *et al.*, 2014). In a study focused on fungi isolated from

varieties of tomato plants that were resistant and susceptible to *Fusarium oxysporum* f. sp. *lycopersic*i (FOL) and were grown in suppressive or non-suppressive soils, Poli *et al.* (2016) demonstrated greater microbial diversity in the rhizosphere than in the root endosphere, and between the two varieties. The rhizosphere mycobiota structure was influenced by soil type, while root endosphere communities were influenced by the plant genotype. The inoculation of FOL modulated the community structure, particularly in the suppressive soil, where *Fusarium* spp. and *Penicillium* spp. were the most responsive fungi. These results confirm data already obtained for bacterial communities (i.e. plant roots select few fungal species from the rhizosphere), and that both soil features and tomato genotype affect fungal communities. In addition, the fungal community structure is differentially influenced by FOL, depending on the suppressiveness or conduciveness of the soil.

Demonstration that some disease-suppressive microbes, such as *Trichoderma*, also compete with AMF (Lace *et al.*, 2015), activating chitinolytic properties and attacking AMF walls, at least under laboratory conditions, opens new unanswered questions on the operational mechanisms of these pathogen competitors. In some suppressive soils, AMF are less abundant than in conducive soils (Chialva *et al.* unpublished). It would be of interest to understand whether soil features, like pathogen suppression, can also negatively affect AMF communities.

Mediterranean crop and root microbiota

The Mediterranean area has been described as one of the most important world biodiversity "hot spots", (Myers *et al.*, 2000), even if examination of microbial diversity in this region has been neglected. In particular, climate model projections identified the Mediterranean region as one of the earth "hot-spots" of climate change, due to the variety of biomes present (Giorgi, 2006; Diffenbaugh and Giorgi, 2012). One of the first predicted outcomes has been identified as a drastic reduction of rainfall and increased temperatures, resulting in reduced availability of water for vegetation, including crops (Pereira, 2011; Saadi *et al.*, 2015). Total crop yield in Mediterranean region is predicted to be reduced by up to 30% in the next 30 years and new management practices are urgently required to overcome these reductions (Saadi *et al.*, 2015). Annual (maize, wheat, soybean, tomato) and perennial (citrus, olive, grapevine) crops are expected to be impacted (Yano *et al.*, 2007; Quiroga and Iglesias, 2009; Patanè and Saita, 2015). Among these, winter wheat and tomato are considered two of the most valuable and strategic herbaceous Mediterranean crops (Saadi *et al.*, 2015). Detailed climate change modelling for tomato and winter wheat yield has recently been done. The study highlighted that even if an expansion of their cultivation range occurs in the next 30 years, climatic risks such as vernalization failure or heat shocks are predicted to increase (Saadi *et al.*, 2015). Both of these crops have been extensively used as models in crop biology research, and their root microbiota has been investigated (Rascovan *et al.*, 2016; Larousse *et al.*, 2017; Tian *et al.*, 2017), as well as for other Mediterranean plants including grapevine and maize (Peiffer *et al.*, 2013; Zarraonaindia *et al.*, 2015; Niu *et al.*, 2017). In contrast, knowledge is limited of the microbiota of other Mediterranean crops, such as citrus and olive.

For environmentally friendly ("sustainable") agriculture, the study of biostimulants has been largely aimed to enhance crop tolerance to biotic or abiotic stresses and climate change effects. AMF have been found to be promising biostimulants (Berruti *et al.*, 2015), and are possibly suitable for promoting crop growth (Geel *et al.*, 2016) in controlled environments. Since most Mediterranean crops are mycorrhizal, the importance of AMF is becoming very relevant. A summary of published research works on AMF and plant microbiota topics is outlined in Figure 4, as well as the use of the major model plant species considered.

Arbuscular mycorrhizal fungi: ubiquitous beneficial components of the plant microbiota

Of the diversity of the fungal world, AMF is one of the most widespread groups, as they are associated with more than 80% of terrestrial plants, including crops (Salvioli and Bonfante, 2013) with which they form arbuscular mycorrhiza (AM) symbiosis. AMF, which are obligate biotrophs, belong to the Glomeromycotina subphylum (Spatafora *et al.*, 2016), which is an ancient clade of basal fungi, related to the Mucoromycotina. Since plants colonized land at least 450 million years ago, Glomeromycotina co-evolved with these plants (Bonfante and Genre, 2008). AMF contribute to the uptake of nutrients from soil to plants, increasing plant growth and conferring resistance to environmental stresses.

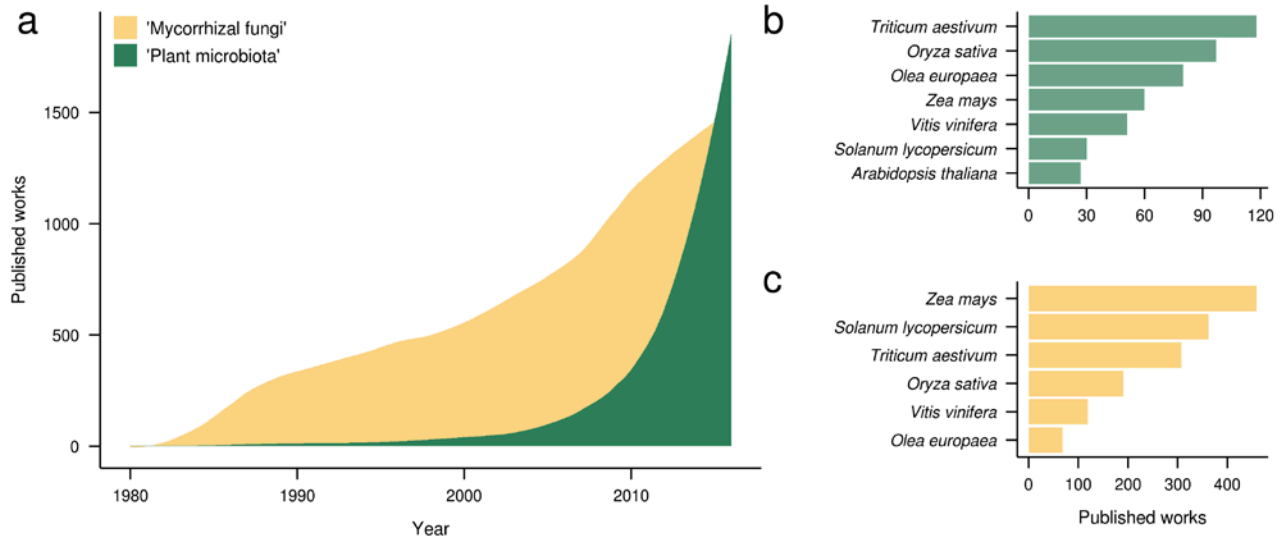

Figure 4. Analysis of literature (last four decades), by 'Plant microbiota' and 'Mycorrhizal fungi' keywords. a, Numbers of published studies on each topic (in green and orange respectively). b-c, Analysis of occurrence in titles by main model and crop species in the Mediterranean region is reported for each topic presented in panel a. Literature was searched by using ISI Web of Science database for the period 1980-2016.

To establish a successful symbiosis, several signalling steps are required between plant and fungal partners. In recent years, also provided by sequencing platforms and molecular techniques, detailed investigations have characterized the molecular interactions occurring during AM symbiosis (reviewed in Gutjahr and Parniske, 2013). Sequencing of the *Rhizophagus irregularis* genome (Tisserant *et al.*, 2013), the first Glomeromycotina genome available, with the addition of several other AMF transcriptomes (Salvioli *et al.*, 2016; Tang *et al.*, 2016), has opened new horizons for AMF research.

Extensive networks of extraradical mycelium (ERM) ensure extensive soil exploration providing plants with low-available mineral resources, such as phosphorous (reviewed in Giovannetti *et al.*, 2017). AMF allow optimal exploitation of environmental trophic resources, helping their plant hosts in the uptake of other soil nutrients including N, S, K, Ca, Fe, Cu and Zn (Smith and Read, 2008). As a result, AMF increase plant biomass and confer improved resistance to pathogens and environmental stresses such as drought and pollutants. In exchange, plants provide AMF with organic carbon sources in the form of lipids (Bravo *et al.*, 2017; Jiang *et al.*, 2017; Keymer *et al.*, 2017), permitting hyphal growth. This mutualistic symbiosis allows an efficient horizontal transfer of nutrients and assists carbon cycling across the atmosphere and biosphere through the mobilization of ~20% of plant photosynthesis products (Harrison, 2005). The ERM network of AMF also improves soil structure, thus increasing the amounts of water-stable aggregates (Degens *et al.*, 1996).

Since AM symbiosis plays important roles in ecosystem and biome dynamics, participating in intricate trophic webs (known as "wood-wide-webs" (Helgason *et al.*, 1998), knowledge of the dynamics of AMF communities as components of plant microbiota, and their impacts on host plant physiology, provides important understanding of factors affecting plant growth and productivity.

Distribution and diversity of arbuscular mycorrhizal fungi

Due to their ecological roles in nutrient cycling and their impacts on plant health (Van Der Heijden and Scheublin, 2007; Johnson *et al.*, 2016), the distribution of AMF has been widely investigated. However, since AMF are obligate plant root symbionts and cannot be cultured, knowledge of their ecology has largely relied on the use of DNA-based identification,

taking advantage of NGS (Hart *et al.*, 2015). NGS-related biases (Lindahl *et al.*, 2013) particularly apply when investigating AMF diversity, because many aspects of the biology and genetics remain unknown (Hart *et al.*, 2015). Notwithstanding these technical limitations, AMF diversity has been described in diverse environments by considering both global and local scales. Davison and co-workers (2015) investigated AMF rDNA from plant roots collected from worldwide sources to study their distribution. AMF communities mirrored local environmental conditions and the geographic distance between sampling sites. They found that 93% of taxa occurred on more than one continent but only 34% on all six world continents studied. This differs from the high spatial singularity of many other fungal taxa and with host plant endemism on the global scale (Davison *et al.*, 2015). AMF probably have dispersal abilities that are more efficient than expected, probably due to biotic and abiotic vectors. At the local scale, diverse environments have been considered, including disturbed and stressed environments (Lekberg *et al.*, 2012), or in mountain vineyards (Berruti *et al.*, 2017). Other examples are protected locations such as exotic camellias of Japanese origin living in Lake Maggiore (Italy) sites (Borriello *et al.*, 2015), and AMF associated with *Festuca brevipila* in semi-arid grassland characterized by high plant diversity and sharp soil gradients in pH, C, N, P (Horn *et al.*, 2014). The latter study revealed how spatial and plant species are a major source of variation for AMF communities at small scale (1–10 m). The data reinforce the concept that understanding of AMF ecology requires knowledge of their biological traits (Horn *et al.*, 2014).

Impacts of arbuscular mycorrhizal fungi on plant hosts: tomato as a Mediterranean crop and model plant in mycorrhiza studies

Even though tomato represents a crucial model crop species, its microbiota has been poorly investigated by using both culture-dependent and -independent methods (Ofek *et al.*, 2014; Rosberg *et al.*, 2014; Lee *et al.*, 2016; Poli *et al.*, 2016; Larousse *et al.*, 2017; Tian *et al.*, 2017). As a matter of fact these studies only investigated tomato rhizosphere (see Table 1), whilst root endosphere communities remain largely unknown.

Tomato was one of the first non-legume models to be used in AMF studies, since it responds to mycorrhizal colonization (Fiorilli *et al.*, 2009; Salvioli *et al.*, 2012; Zouari *et al.*, 2014), native microbiota (Chialva *et al.*, 2018) and provides a useful tool to investigate metabolic processes and their relation to gene expression. Tomato avoids the overlapping signalling transduction pathway, since it does not establish the *Rhizobium*-AMF tripartite symbiosis that occurs in legumes (Barker *et al.*, 1998), thus permitting additional information concerning the non common symbiosis signalling pathway (CSP).

Tomato mutants, are also widely used in the study of AM symbiosis functioning, and have offered important clues for mycorrhizal phenotypes related to differences in hormonal control. The first non-legume mutant impaired in AM colonization was characterized by Barker *et al.* (1998) from tomato, the reduced mycorrhizal colonization (*rmc*) genotype. The mutation blocks AMF colonization at early stages with different mycorrhizal phenotypes depending on the AMF species (Gao *et al.*, 2001). Other tomato mutants unable to form AM symbiosis were further characterized and named pre-mycorrhizal infection (*pmi1* and *pmi2* mutants), since they are blocked at the pre-colonization stages (David-Schwartz *et al.*, 2003). Mutations inhibited hyphal branching and spore germination but did not interfere in plant-host signalling (Gadkar *et al.*, 2003). Tomato mutants were also used to determine the role of hormonal metabolism in AM establishment. As an example, fruit ripening affected mutants have been used in these studies (Torres de Los Santos *et al.*, 2011).

Further attention has been given to understanding AMF impacts on tomato biology in shoots and above ground organs. Several tomato plant traits are reported to be positively affected by AMF colonization. Mycorrhizal colonization can increase root dry weight (Bryla and Koide, 1998), plant height, shoot dry weight (Bryla and Koide, 1998; Utkhede, 2006; Copetta *et al.*, 2011; Latef and Chaoxing, 2011) and leaf area (Poulton *et al.*, 2002). Tomato is also a good model for AMF and fruit value-enhancing research. Several authors have reported increased fruit yields from AMF colonization both in greenhouse conditions (Utkhede, 2006; Dasgan *et al.*, 2008) and in field-grown plants (Plenchette *et al.*, 1983; Mohandas, 1987; Regvar *et al.*, 2003). Regvar *et al.* (2003) suggested these effects are related to extended fruiting periods.

Considering nutraceutics in fruits, Copetta *et al.* (2011) detected greater concentrations of fructose and

glucose in tomato fruit from mycorrhizal plants. Other studies have suggested that AMF also increase fruit antioxidant compounds, as carotenoids (β-carotene and lycopene) (Regvar *et al.*, 2003) and human health-enhancing compounds such as phenolics (Ulrichs *et al.*, 2008). These metabolomic responses to AMF colonization are related to the farming practices: the maximum amounts of these compounds occurred in inoculated tomato plants grown organically (fertilized with compost). In this scenario, a synergistic effect by AMF and compost microbiota should also be possible but the topic needs to be investigated. New evidence shows how tomato fruit from mycorrhizal plants are enriched in compounds such as lycopene, and are safe for consumers because of anti-oestrogenic capacity without *in vitro* genotoxic effects (Giovannetti *et al.*, 2012). Tomato fruits from mycorrhizal plants have increased amounts of amino acids content (Salvioli *et al.*, 2012), are enriched in ascorbic acid (vitamin C) and total soluble solids (Subramanian *et al.*, 2006). These beneficial effects of AMF at vegetative and reproductive levels are probably related to improved plant phosphate nutrition, since similar effects in the reproductive phenology were reported under a high phosphate treatment (Poulton *et al.*, 2002).

New high throughput techniques have permitted cataloguing of the molecular re-programming that occurs during mycorrhizal symbiosis. A consistent set of mycorrhiza-induced genes was described as regulating nutrient transport and plant development, or responses to abiotic and biotic stimuli in roots. Extending this analysis to whole plants, the existence of a gene core was assessed, modulated independently from particular organs and induced by mycorrhiza formation, as well as a systemic defence-related gene programme in shoots (Salvioli and Bonfante, 2013). These data suggest that an organism-wide reprogramming is activated by tomato under mycorrhizal conditions. Despite this premise, only a few studies have shown the transcriptional basis of these processes. Schwarz *et al.* (2011) showed how mycorrhiza-induced systemic effects result in an increased expression of allergen-encoding genes that does not correlate with an increased allergenic potential for humans. Salvioli *et al.* (2012) confirmed a systemic AMF effect ("MYC-effect"), with the differential regulation of eleven transcripts in tomato fruit, using TOM2 microarray and Real-Time PCR techniques. Several metabolic pathways of tomato fruit were regulated, involving aroma components and fruit development. However, due to limited genome-coverage of the TOM2 microarray, other transcript regulations are possible. To complete the molecular framework on fruits from mycorrhizal plants, tomato transcriptome analysis has been performed with the Illumina sequencing technology (Zouari *et al.*, 2014). Preliminary data have highlighted a differential expression of 712 sequences between mycorrhizal (MYC) and fertilized plants, most of which were up-regulated in the MYC condition. Induced transcripts refer principally to photosynthesis, stress responses, amino acid synthesis and transport, carbohydrates metabolism and photorespiration, validating the results previous obtained by Salvioli *et al.* (2012). Regulation of primary metabolism processes may suggest that AM affect source-sink dynamics. In contrast, cell wall metabolic pathways were downregulated, and included enzymes such as the polygalacturonase (PGA) involved in fruit ripening. Other evidences, including the involvement of ethylene metabolism, suggest that the mycorrhizal condition may increase fruit shelf-life. For the first time, a fruit systemic "signature" was assessed at the transcriptomic level.

Our previous studies have demonstrated that, among a panel of selected tomato ripening mutants, two mutations involved in ethylene (*Gr*) and light perception (*hp-1*) have impacts on mycorrhiza functioning, as well as in systemic-induced fruit and plant traits, but not on root mycorrhizal phenotype (Chialva *et al.*, 2016). These data reveal that the plant responses to arbuscular mycorrhiza are evolutionarily well-separated from the molecular re-programming involved in mycorrhiza functioning at the host root level.

Taken as a whole, these data demonstrate that tomato is an AMF responsive plant both at root and systemic levels. However, not all the data indicate similar effects. For example, lycopene enrichment (Giovannetti *et al.*, 2012) is not supported by transcriptomic data (Zouari *et al.*, 2014), and results from applied phosphate suggest that our knowledge of P availability, tomato growth and molecular responses to AMF is not complete.

Conclusions

The increasing demand for safe and healthy food has recently drawn attention to low energy input and low impact sustainable agriculture, and several international initiatives promoted by government (e.g. several EU Horizon 2020 project calls) or by private

foundations (e.g. The Bill and Melinda Gates foundation) have addressed these issues. The most recent data on plant microbiota reveal that this is a key challenge for plant and agricultural sciences: to develop safe plants for safe food. Many efforts have been devoted to understanding the biodiversity of microbes living in soil and associated with plant organs. Moving from identification to metagenomic and functional analyses is allowing description of the functionality of such microbial communities and their effects on host physiology, although this step is still in its infancy. The increasing knowledge on plant microbiota has already led to the introduction of new concepts and new terminologies that are opening future theoretical and practical research fields.

Acknowledgement

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Glossary and acronyms

Arbuscular mycorrhizal fungi (**AMF**): group of soil fungi belonging to the Glomeromycotina clade (phylum Mucoromycota) living in the roots of most land plants with which they form, as obligate symbionts, the Arbuscular Mycorrhiza (**AM**) symbiosis.

Common Symbiotic Pathway (**CSP)**: a signal transduction pathway, transducing signals released by Glomeromycotina and Rhizobia from plasma membrane receptors into the nucleus of plants susceptible to root symbioses.

Core root microbiome: Common members to two or more microbial assemblages associated with a habitat (reviewed in Shade and Handelsman (2012)). Current hypotheses claim that a core root microbiome has evolved with terrestrial plants over their 400 million year history.

Disease-suppressive soils: can reduce pathogenic attacks to plants thanks to specific features of their microbial composition.

Ectomycorrhizal fungi (**ECM**): group of soil fungi mostly belonging to Asco- and Basidiomycota, and living associated to the roots of many woody plants, as symbionts.

Endorhiza: defines a compartment hosted into plant tissues.

Extraradical mycelium (**ERM**): the complex network of AMF and ECM hyphae, which grow in the rhizosphere, develop propagules, and acquire nutrients, at the basis of the symbiotic exchanges with the host plants.

Holobionts (**meta-organisms**): the assemblage of the host plus of all its microbial symbionts

Hologenome: the full set of the host genome plus its microbiome.

Induced systemic resistance (**ISR**): induced state of resistance which is triggered by biological or chemical inducers. It may protect nonexposed plant parts against future attack by pathogenic microbes and herbivorous insects.

Lipochitooligosaccharides (**LCOs**) and **Chitooligosaccharides** (**COs**): chitin-derived molecules released by Arbuscular mycorrhizal fungi and acting as bioactive signalling molecules

Metagenome: the full set of genomes present in a microbial habitat (microbiota), considered as a unique entity at the moment of sampling.

Metatranscriptome: the total content of gene transcripts in a microbial community (microbiota), considered as a unique entity at the moment of sampling.

Microbiota: complex microbial assemblage that lives in a given environment or niche.

Mycorrhizal fungi: the term covers a huge variety of different soil fungi which live associated to the roots of land plants with very diverse morphologies and functional features.

Next-Generation Sequencing (**NGS**) techniques: term used to describe a number of different high-throughput DNA/RNA sequencing technologies (HTS) (see BOX in the text).

Plant growth-promoting rhizobacteria (**PGPRs**): bacteria that colonize plant roots enhancing plant growth by solubilizing soil nutrients, producing phytormones or anti-microbial compounds, or inducing the systemic resistance in the plant.

Phyllosphere: the epigeous portions of plants as habitat for microorganisms. It can be subdivided into the caulosphere (stems), phylloplane (leaves), anthosphere (flowers), and carposphere (fruits) and spermosphere (seeds).

Plant immune system: protects plants from pathogens by pre-formed structures and chemicals, and by infection-induced responses. The latter require a complex network of receptors which sense microbial molecules outside and inside plant cells activating antimicrobial defenses.

Rhizosphere: the soil area that is directly influenced by root secretions and associated soil microorganisms.

Rhizoplane: the root surface with closely adhering soil particles and debris.

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